

IV. WHAT IS CLAIMED IS:

1 1. A method of processing collagen-based tissue prior to implantation into a
2 recipient in need thereof, said method comprising the steps of:
3 decellurizing said collagen-based tissue such that substantially all cells, cellular
4 debris, lipids and proteins are removed; and
5 preserving the resulting collagen scaffold through a bioreactor, cryopreservation,
6 freezing, chilling, drying, room temperature packaging, or freeze-drying.

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1 2. The method of processing collagen-based tissue prior to implantation into a
2 recipient in need thereof, according to claim 1, further comprising repopulating the
3 collagen scaffold with cells having lower immunogenicity toward the recipient than the
4 collagen-based tissue; and growing said cells on and within said collagen-based tissue in
5 an organ perfusion system.

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1 3. An acellular collagen-based tissue produced according to the method of claim 1.

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1 4. The method according to claim 1, wherein said collagen-based tissue is selected
2 from the group consisting of a heart, heart valve, joint, soft tissue organ and vasculature.

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1 5. The method according to claim 1, wherein said collagen-based tissue consists of a
2 total joint.

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1 6. The method according to claim 1, wherein said collagen-based tissue consists of a
2 trachea.

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1 7. The method according to claim 1, wherein said collagen-based tissue consists of a
2 knee, shoulder, wrist, ankle or elbow joint.

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1 8. A method of replacing collagen-based tissue with a processed collagen-based
2 tissue in a recipient in need thereof which comprises implanting acellular collagen-based
3 tissue or acellular collagen-based tissue repopulated with cells into said recipient.

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1 9. An implant cleaning, perfusion and passivation process which comprises cyclic
2 exposure of said implant to increased and decreased positive or negative pressures, or
3 both.

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1 10. An apparatus for conducting the process according to claim 9 comprising a
2 reaction chamber **120**, said reaction chamber **120** in communication with an air piston
3 **110**, such that increased pressure and decreased positive or negative pressures, or both,
4 via movement of the air piston **110**.

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1 11. The apparatus of claim 10 wherein said air piston **110** and said reaction chamber
2 **120** are connected via a conduit **128**.

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1 12. The apparatus of claim 12, wherein said apparatus comprises a filter **122** along the
2 conduit **128**.

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13. A method for treating and processing tissue for implantation that decellularizes and
inactivates virus in said tissue comprising the steps of:

1 a) contacting said tissue with a viral inactivating agent, wherein said viral
2 inactivating agent comprises benzalkonium chloride; and

5 b) contacting said tissue with a decellularizing agent;

whereby said tissue maintains structural integrity and activity of growth factors in said
tissue is maintained.

14. The method of claim 13, wherein said viral inactivating agent comprises about 0.5
10 percent or more, weight percent, benzalkonium chloride solution.

15. The method of claim 14, wherein said viral inactivating agent comprises about 0.5 percent, weight percent, benzalkonium chloride solution.

16. The method of claim 13, wherein said decellularizing agent comprises a solution comprising, by weight, about 0.5 percent or more Tween 20 and about 0.5 percent or more hydrogen peroxide.

17. The method of claim 16, wherein said decellularizing agent comprises about 1 percent Tween 20 and about 0.5 percent hydrogen peroxide, and wherein said tissue is sonicated during contact with said decellularizing agent.

18. The method of claim 13, wherein said tissue is bone, neural tissue, fibrous connective tissue including tendons and ligaments, cartilage, dura, pericardia, muscle, heart valves, veins and arteries and other vasculature, dermis, adipose tissue, or glandular tissue.

19. The method of claim 18 wherein said tissue is bone, heart valve(s), vein(s), tendon, ligament or dermis.

20. The method of claim 13 wherein said tissue is dermis.

21. A method of decellularizing and viral inactivating tissue comprising the steps of:

a) contacting said tissue with a viral inactivating agent; and
b) contacting said tissue with a decellularizing agent, wherein said decellularizing agent comprises a solution comprising, by weight, about 0.5 percent or more Tween 20 and about 0.5 percent or more hydrogen peroxide.

22. The method of claim 21 wherein said decellularizing agent comprises a solution comprising, by weight, about 1 percent Tween 20 and about 0.5 percent hydrogen peroxide; and wherein said method further optionally comprises sonicating said tissue during step b.

23. The method of claim 21 wherein said tissue is dermis.

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24. A method for treating tissue effecting the decellularizing and inactivating viruses in said tissue comprising the steps of:

- a) contacting said tissue with a solution comprising about 0.5 percent or more, by weight, benzalkonium chloride;
- 50 b) contacting said tissue with a solution comprising about 0.5 percent or more, by weight, tween 20 and about 0.5 percent or more, by weight, hydrogen peroxide; and
- c) contacting said tissue with a calcium hydroxide solution.

25. The method of claim 24, wherein said calcium hydroxide solution is saturated.

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26. The method of claim 24, further comprising contacting said tissue treated with said calcium hydroxide solution with a calcium chelating agent; optionally sonicating said tissue during contacting said tissue with said chelating agent.

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27. The method of claim 26, wherein said calcium chelating agent is a solution comprising about 0.5 percent to about 5 percent EDTA.

28. The method of claim 24, further comprising drying said tissue.

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29. The method of claim 28 wherein drying said tissue comprises contacting said tissue with an alcohol solution.

30. The method of claim 24, further comprising lyophilizing said tissue.

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31. The method of claim 24, further comprising cutting and packaging said tissue.

32. The method of claim 24, wherein said tissue is sonicated during steps b and c.

33. The method of claim 32 further comprising irradiating said tissue:

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34. A method for decellularizing and inactivating viruses in dermis tissue comprising the steps of:

- a) obtaining a sample of crude dermis tissue;
- b) treating said crude dermis tissue with sodium chloride;
- 80 c) separating epidermis from dermis of said crude dermis tissue by manual debridement to produce dermis sample;
- d) contacting said dermis sample with a solution comprising 0.5 percent or more, by weight, benzalkonium chloride;
- e) contacting said dermis sample with a solution comprising 0.5 percent or more, 85 by weight, tween 20 and 0.5 percent or more hydrogen peroxide; optionally further comprising simultaneous sonication of said dermis sample;
- f) contacting said dermis with a solution of saturated calcium hydroxide; and subsequent rinsing of said dermis sample followed by chelating of said dermis sample by contact with a chelating agent; and optionally further comprising sonicating said dermis sample during contact with said saturated calcium hydroxide;
- 90 g) neutralizing pH of said dermis sample with a neutralizing buffer, followed by rinsing said dermis sample
- h) drying said dermis sample with an alcohol solution comprising about 50 to about 100 percent, by weight, alcohol;
- 95 i) lyophilizing said dermis sample;
- j) cutting said dermis sample; and
- k) irradiating said dermis sample.